[illegible] and may simply be because researchers have routinely used molybdenum-containing media to isolate N_2-fixing bacteria. The other nitrogenases may be quite common in nature, though this possibility has not yet been fully explored. The study of alternative nitrogenase systems is quite recent, and many fundamental questions remain unanswered.

The Genetics of Nitrogenase

As one might surmise from the complexities of the N_2-fixing system, the genetics of the process are equally complex. Much of our knowledge of the genetics of the diazotrophic system has come from the intensive study of the bacterium *Klebsiella pneumoniae*, a member of the family Enterobacteriaceae and a close relative of *Escherichia coli*. Twenty-five years of research on this bacterium and, more recently, other diazotrophic bacteria have shown that the nitrogenase complex and supporting systems are under the control of no less than 21 genes. Because of this complexity, the system does not lend itself to easy genetic manipulation and transfer to higher organisms. The prospect of genetically engineering crops like corn to fix their own nitrogen remains an elusive goal for researchers.

The Free-Living Dinitrogen-Fixing Bacteria

Biological N_2 fixation is restricted to prokaryotes. This includes typical bacteria and specialized bacteria like cyanobacteria and the actinomycetes. Of the 10,000 or so bacterial genera named, only about 100 contain bonafide diazotrophic species. Although this may appear to be a somewhat limited representation, species representing all sorts of physiological types and occupying all sorts of ecological niches have been described. Some of the genera of free-living diazotrophs are listed in Table 13.4. These organisms encompass such groups as heterotrophic and chemoautotrophic bacteria, photoautotrophs (bacteria and cyanobacteria), and photoheterotrophs with respect to carbon metabolism. Note also that they are well represented by aerobes, **microaerophiles** that grow best at low oxygen tension, facultative anaerobes, and obligate anaerobes. Such metabolic diversity enables some type of diazotroph to colonize almost any imaginable sort of habitat. Indeed, diazotrophs are widespread in nature as free-living microbes and in a large number of associations with plants and animals. This great metabolic diversity means that in all sorts of environments, diazotrophs can make contributions to the supply of fixed nitrogen for growth of nonfixing microbes and higher forms of life. For example, diazotrophs colonize the roots and rhizospheres of many plant species and make small quantities of fixed nitrogen available to the plants.

Factors Affecting Dinitrogen Fixation by Free-Living Diazotrophs

Thus far, we have discussed the complexities and uniqueness of the nitrogenase complex. In this section the factors that must be successfully integrated for a nonsymbiotic diazotroph to fix dinitrogen will be discussed, followed by a description of the integration of these factors with respect to the functioning of nonsymbiotic diazotrophs in association with higher plants, the so-called **associative symbioses.**

Table 13–4 List of genera of microbes which include free-living, N_2-fixing species or strains. This list is not intended to be all-inclusive.

	Genus or type	Species (examples only)
Aerobes	*Azotobacter*	*A. chroococcum**, *A. vinelandii**
	Azotococcus	*A. agilis**
	Azomonas	*A. macrocytogenes**
	Beijerinckia	*B. indica**, *B. fluminis**
	Derxia	*D. gummosa**
	Pseudomonas	*P. stutzeri*, *P. saccharophila*
	Azoarcus	*A. communis*, *A. indigens*
	Acetobacter	*A. diazotrophicus*
Facultative (aerobic when not fixing N_2)	*Klebsiella*	*K. pneumoniae*, *K. oxytoca*
	Bacillus	*B. polymyxa*, *B. macerans*
	Enterobacter	*E. agglomerans* (*Erwinia herbicola*)
	Citrobacter	*C. freundii*
	Escherichia	*E. intermedia*
	Propionibacterium	*P. shermanii*, *P. petersonii*
Microaerophiles (normal aerobes when not fixing N_2)	*Xanthobacter*	*X. flavus**, *X. autotrophicus*
	Thiobacillus	*T. ferro-oxidans*
	Azospirillum	*A. lipoferum**, *A. brasilense**
	Aquaspirillum	*A. perigrinum**, *A. fascicilus**
	Methylosinus	*M. trichosporum*
Strict anaerobes	*Clostridium*	*C. pasteurianum**, *C. butyricum*
	Desulfovibrio	*D. vulgaris*, *D. desulficuricans*
	Methanosarcina	*M. barkeri*
Phototrophs (aerobic) cyanobacteria	*Anabaena*	*A. cylindrica*, *A. inaequalis*
	Nostoc	*N. muscorum*
	Calothrix	
	(7 other genera of heterocystous cyanobacteria)	
	Gloeothece	*G. alpicola*
Phototrophs (microaerophiles) cyanobacteria	*Plectonema*	*P. boryanum*
	Lyngbya	*L. aestuarii*
	Oscillatoria	
	Spirulina	
Phototrophs (facultative) bacteria	*Rhodospirillum*	*R. rubrum*
	Rhodopseudomonas	*R. palustris*
Phototrophs (anaerobes) bacteria	*Chromatium*	*C. vinosum*
	Chlorobium	*C. limicola*
	Thiopedia	
	Ectothiospira	*E. shapovnikovii*

Adapted from Postgate (1987). Used with permission. See also Young (1992).
*Signifies that all reported strains of the species fix dinitrogen.

Sources of Energy for Diazotrophs

With the exception of the phototrophic bacteria and cyanobacteria, all diazotrophs require an organic or inorganic, in the case of chemoautotrophs, energy source. A requirement for abundant energy sources is dictated by the high energy demands of nitrogenase. Remember, a minimum of 16 ATPs are required to make two molecules of ammonia from dinitrogen. A wide variety of carbon sources, ranging from methane (CH_4) to complex carbohydrates, can be used by one diazotroph or another. It is not usually the type of carbon source that limits dinitrogen fixation, but the lack of an ample supply in many habitats that limits fixation by the nonsymbiotic bacteria. The soil is not an "organic soup" abundantly supplied with readily available carbon sources, and diazotrophs must compete with all the other soil microbes for the same carbon.

In terms of carbon sources for energy, it is interesting to consider the efficiency of N_2 fixation according to the amount of carbon consumed per nitrogen fixed (Table 13–5). Note that the efficiencies of N_2 fixation in terms of mg of N fixed g^{-1} of carbon source are low, averaging about 15 mg N g^{-1} of carbon source. Also, the assimilation of ammonium is about twice as efficient as N_2 fixation, and that fixation by anaerobes is generally much less efficient than by aerobes. An exception to this

Table 13–5 **Carbon and energy source requirements for heterotrophs grown in chemostats limited by the carbon and energy source.**

Organism	Carbon and energy source	Efficiency of nitrogen incorporation during growth (mg N g^{-1} C and energy source used)		Carbon and energy expenditure for N_2 fixation	
		N_2	NH_4^+	($g\ C\ g^{-1}\ N_2$)	lb C used per 100 lb of N_2 fixed
Anaerobic growth by fermentation					
Clostridium pasteurianum	Sucrose	11	22	46	4,600 (2,054)*
Klebsiella pneumoniae	Glucose	8	19	72	7,200 (3,214)
Aerobic growth					
Klebsiella pneumoniae (O_2-limited)	Glucose	15	35	38	3,800 (1,696)
Azospirillum brasilense (9 μM O_2)	Malate	26	48	19	1,900 (848)
Azotobacter vinelandii (2–10 μM O_2)	Sucrose	16	63	47	4,700 (2,098)
Azotobacter vinelandii (180 μM O_2)	Sucrose	7	38	117	11,700 (5,223)

Adapted from Hill (1992). Used with permission. See also Giller and Day (1985).

*The 100-lb figure is chosen as representative of a typical amount of fertilizer nitrogen that might be applied to a crop. Numbers in ( ) are values in kg.

From one mole of Nitrogen gas two moles of ammonia are produced.

Inorder for nitrogen to be used for growth it must be fixed (combined) in the form of Ammonium (NH_4^+) or Nitrate (NO_3^-) ions

<u>Microbial Biomass nitrogen</u> (MBN):- Different nitrogen sources affect MBN. Org nitrogen (protein) / sources increases Microbial biomass Nitrogen

pH = A soil pH near 7 is most suitable for microbial growth.

Cyanobacteria (Anabaena)

Heterocyst = It is formed in response to lack of fixed nitrogen (NH_4^+ or NO_3^-)

<u>Actinomycetes</u>:- Frankia is a genus of soil actinomycetes. It fixes N both under symbiotic & free living aerobic conditions. These are gram positive bacteria

Vesicle = They have lipid encapsulated vesicles. These vesicles contain Nitrogenase
= Liquid or air filled swelling or sac.